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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C12N 5/10, C12P 21/00
A61K 37/00, 39/00

A1

(11) International Publication Number: WO 93/10220

(43) International Publication Date: 27 May 1993 (27.05.93)

(21) International Application Number: PCT/US92/10030

(22) International Filing Date: 18 November 1992 (18.11.92)

(30) Priority data:
07/795,897 19 November 1991 (19.11.91) US

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(81) Designated States: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG).

Published
*With international search report.*

(54) Title: SOLUBLE MHC MOLECULES AND THEIR USES

(57) Abstract

The present invention provides novel chimeric proteins comprising an MHC component linked to an immunoglobulin constant region component, wherein the chimeric protein is capable of selectively binding a T cell receptor through the MHC component. The claimed chimeric molecules may also comprise a protease recognition site between the two components to facilitate purification of either component. The chimerics are useful in treating MHC associated disorders, such as autoimmune diseases.

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# SOLUBLE MHC MOLECULES AND THEIR USES

## BACKGROUND OF THE INVENTION

The present invention relates generally to chimeric proteins and, in particular, to chimeric proteins comprising an MHC component and an immunoglobulin constant region component. The chimeric molecules specifically bind T cell receptors through the MHC component while retaining desired functions of the constant region component.

There is currently a great interest in developing pharmaceuticals based on the growing understanding of the structure and function of the major histocompatibility complex (MHC) antigens. These cell surface glycoproteins are known to play an important role in antigen presentation and in eliciting a variety of T cell responses to antigens.

T cells, unlike B cells, do not directly recognize antigens. Instead, an accessory cell must first process the antigen and present it in association with an MHC molecule in order to elicit an immunological response. The major function of MHC glycoproteins appears to be the binding and presentation of processed antigen in the form of short (10-20 amino acids in length) antigenic peptides.

In addition to binding antigenic peptides, MHC molecules can also bind "self" peptides. If T lymphocytes then respond to cells presenting "self" or autoantigenic peptides, a condition of autoimmunity results. Over 30 autoimmune diseases are presently known, including myasthenia gravis (MG), multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), insulin-dependent diabetes mellitus (IDDM), etc. Characteristic of these diseases is an attack by the immune system on the tissues of the victim. In non-diseased individuals, such attack does not occur because the immune system recognizes these tissues as "self". Autoimmunity results when the ability to recognize certain autoantigens as "self" is lacking.

Current treatment for autoimmune disease and related conditions consists primarily of treating the symptoms, but not intervening in the etiology of the disease. Broad spectrum chemotherapeutic agents are typically employed, which agents are often associated with numerous undesirable side effects. Compounds capable of selectively suppressing autoimmune responses by blocking MHC binding provide a safer, more effective treatment. In addition, such selective immunosuppressive compounds are useful in the treatment of non-autoimmune diseases, such as graft-versus host disease (GVHD) or various allergic responses. For instance, chronic GVHD patients frequently present conditions and symptoms similar to certain autoimmune diseases.

The inadequate treatments presently available illustrate the urgent need to identify new agents that block MHC-restricted immune responses, but avoid undesirable side effects such as nonspecific suppression of an individual's overall immune response. A desirable approach to treating autoimmune diseases and other pathological conditions mediated by MHC is to use antagonists to block binding to the T cell receptor.

MHC molecules themselves can be used as antagonists to therapeutically block the binding of particular T cells and antigen presenting cells. In addition, the molecules can induce anergy, or proliferative noresponsiveness, in targeted T cells. Production of soluble MHC molecules for this purpose would be particularly desirable. The use of soluble MHC as a therapeutic is promising, although a number of improvements in this approach can be made. For instance, improvement of the relatively short serum half-life of soluble MHC molecules is desirable. In addition, the therapeutic effectiveness of these molecules can be increased if they can be modified to be able to cross the placental or other biological barriers. Finally, the ability to eliminate target cells with out the use of toxins would be advantageous.

The prior art thus lacks a soluble agent capable of blocking MHC restricted T cell activation, which agent inter

alia has an extended serum half-life and provides a mechanism for eliminating targeted T cells without the necessity of using t xic conjugates.

## SUMMARY OF THE INVENTION

The present invention provides soluble chimeric proteins comprising an MHC component linked to an immunoglobulin constant region component. The chimeric protein is capable of binding a T cell receptor through the MHC component, while the immunoglobulin component retains normal effector functions and provides the chimeric proteins with an extended serum half-life and other advantages.

The MHC component preferably consists of the extracellular region of a full length MHC glycoprotein. Typically, MHC class II glycoproteins are used. If the chimeric protein is used to treat autoimmune disease, the MHC component will include an autoantigenic peptide associated with the autoimmune disease.

The immunoglobulin constant region component is preferably of the human IgG1 isotype. The immunoglobulin component preferably retains certain effector functions such as the ability to fix complement or to mediate antibody dependent cell cytotoxicity.

To facilitate separation of the two components from each other, the chimeric proteins of the present invention may contain a protease recognition site between the MHC component and the immunoglobulin component. The protease recognition site is preferably one that is not present in the MHC component. Examples of suitable proteases include, Factor Xa and collagenase. Methods for purifying the soluble MHC component using the protease recognition site are also provided.

The present invention further provides pharmaceutical compositions and methods suitable for treating a patient with autoimmune disease. The compositions can also be used for a variety of diagnostic purposes, as well.

The invention provid s rec mbinant expression cassettes which are incorporated in expression vectors and used to transf rm a variety of host cells. Methods are discl sed for transforming appropriate host cells and recovering the expressed chimeric proteins from the cell culture.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic representation of an MHC molecule showing the extracellular, the transmembrane, and cytoplasmic regions.

FIG. 2 is a schematic of a a MHC class II/immunoglobulin chimeric molecule of the present invention.

FIG. 3 is a schematic of a $\alpha$ homodimeric molecule of the present invention comprising two chains, each comprising an MHC II $\alpha$ chain attached to the immunoglobulin constant region.

FIG. 4 is a schematic of a $\alpha+\beta$ heavy chain homodimeric molecule of the present invention comprising an MHC II $\alpha$ chain attached to the immunoglobulin constant region and a MHC II $\beta$ chain linked to the $\alpha$ chain.

FIGS. 5a and 5b show two expression vectors encoding chimeric proteins of the present invention. FIG. 5a shows an expression cassette comprising a cDNA segment encoding the immunoglobulin component. FIG. 5b shows an expression cassette comprising a genomic DNA segment encoding the immunoglobulin component.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

The chimeric proteins of the present invention comprise a major histocompatibility complex (MHC) component linked to an immunoglobulin constant region component. The MHC component allows the chimeric protein to bind a T cell receptor, while the immunoglobulin component provides, inter alia, increased serum half-life and various effector functions. The chimeric proteins may also comprise a protease recognition site between the two components. Treatment of the chimeric protein with an appr priate protease all ws purification of the MHC component, if, f r instance, the chimeric protein is bound

to an affinity chromatography column through the immunoglobulin component. These and other advantages of the chimeric proteins of the pr sent invention will become apparent from the following description of the components of the claimed proteins.

The MHC component of the chimeric proteins determines the specificity of the proteins by binding selected T cell receptors. The glycoproteins encoded by the MHC have been extensively studied in both the human and murine systems. They are classified according to the kinds of cells on which they are expressed and the T cells which recognize them. Class I MHC molecules (e.g., HLA-A, -B and -C molecules in the human system) are expressed on almost all nucleated cells and are recognized by cytotoxic T lymphocytes, which then destroy the antigen-bearing cells. Class II MHC molecules (HLA-DP, -DQ and -DR in humans) are expressed primarily on antigen presenting cells such as B lymphocytes, macrophages, etc. This class of MHC molecules is recognized by helper T lymphocytes and induces proliferation of both B and T lymphocytes, thus amplifying the immune response to the particular antigenic peptide that is displayed. MHC glycoproteins of both classes have been isol ed and characterized (see, Fundamental Immunology, 2d Ed., W.E. Paul ed., Ravens Press, N.Y., (1989), and Roitt, et al., Immunology, 2d Ed., Gower Medical Publishing, London, (1989) which are both incorporated herein by reference).

After the antigenic material is processed by the MHC-bearing cell, the resulting antigenic peptide first forms a complex with the antigen binding pocket of the MHC molecule through various noncovalent associations. This complex then fits into a single recognition site in a T cell receptor on a cytotoxic or helper T cell, depending upon the class of MHC molecule. For a general discussion of the function of MHC molecules, see Grey, H.M., et al., Scientific American pp 56-64 (November, 1989) which is incorporated herein by reference and Paul, supra, Chapter 18.

M thods for purifying the Class II MHC glycoprot ins are known. See, e.g., Turkewitz, A.P., et al., Molecular

Immunology 20:1139-1147 (1983) , which is incorporated herein by reference. Figure 1 presents a schematic representati n of a Class II MHC molecule. Class II MHC antigens are heterodimeric transmembrane glycoproteins consisting of an $\alpha$ chain (MW 25-33 kD) and a $\beta$ chain (MW 24-29 kD), which are noncovalently associated. Figure 1 shows that each chain consists of two globular domains, a linker peptide, a transmembrane region, and a cytoplasmic tail. Both globular domains of the $\beta$ chain are stabilized by intrachain disulfide bonds, whereas the $\alpha$ chain contains only one such disulfide bond (see, Paul, supra, Chapters 16 and 17).

The human Class I proteins have also been studied. The Class I MHC of humans on chromosome 6 has three loci, HLA-, HLA-B, and HLA-C, the first two of which have a large number of alleles. Class I molecules consist of a 44 kd subunit noncovalently associated with a 12 kd $\beta_2$-microglobulin subunit. $\beta_2$-microglobulin is not encoded by a locus in the MHC but is common to all Class I molecules. Although $\beta_2$-microglobulin does not form part of the antigen binding pocket of the molecule, it is necessary for processing and expression of Class I MHC molecules. Isolation of detergent-soluble HLA antigens has been described by Springer, T.A., et al., Proc. Natl. Acad. Sci. USA 73:2481-2485 (1976); Clementson, K.J., et al., in "Membrane Proteins" Azzi, A., ed; Bjorkman, P., Ph.D. Thesis Harvard (1984) all of which are incorporated herein by reference.

Further work has resulted in a detailed picture of the 3-D structure of HLA-A2, a Class I human antigen. (Bjorkman, P.J., et al., Nature 329:506-512, 512-518 (1987) which are incorporated herein by reference). The $\beta_2$-microglobulin protein is associated with the $\alpha_3$ domain of the heavy chain, while the $\alpha_1$ and $\alpha_2$ domains appear to form the contours of the antigen-binding pocket which contains the selective binding sites of the molecule. (Science 238:613-614 (1987), which is incorporated herein by reference and Bjorkman et al., supra). Soluble HLA-A2 can be purified after papain digestion of plasma membranes from the homozyg us human

lymphoblastoid cell line J-Y as described by Turner, M.J. et al., J. Biol. Chem. 252:7555-7567 (1977), which is incorporated her in by reference. Papain cleaves the 44 kd chain close to the transmembrane region yielding a molecule comprised of $\alpha_1$, $\alpha_2$, $\alpha_3$, and $\beta_2$-microglobulin.

While the three dimensional structure of Class II MHC antigens is not known in such detail, it is thought that Class II glycoproteins have a domain structure, including an antigen binding pocket, similar to that of Class I. The antigen binding pocket is thought to be formed from the N-terminal domain portions of $\alpha$ and $\beta$ chains. Comparison of the sequences of the protein chains from the two classes has revealed regions in the Class II $\beta$ chain with homology to MHC Class I $\alpha_1$ and $\alpha_2$ regions (see, Paul, Chapter 17).

The term "MHC component" as used herein refers to a purified a MHC glycoprotein or portion thereof which is in other than its native state, that is, not associated with the cell membrane of a cell that normally expresses MHC. The "extracellular region" of the molecule is a water soluble portion of the molecule consisting of the sequences extending from the N-terminus to the transmembrane region and comprises the antigen binding pocket as well as other sequences necessary for recognition by the appropriate T cell receptor. The extracellular region may comprise sequences from the transmembrane region (up to about ten amino acids), so long as solubility is not significantly affected.

The second major component of the chimeric proteins of the present invention is the immunoglobulin constant region component. Immunoglobulins are a group of glycoproteins present in the serum and tissue fluids of all mammals. The basic immunoglobulin structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25kD) and one "heavy" chain (about 50-70kD). The N-terminal region of the chains defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The C-terminal region of the chains defines a constant region

primarily responsible for effector function. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. (see, Paul and Roitt et al., supra).

Within the light and heavy chain, units made up of about 110 amino acids form discrete domains. Each domain is held together by a single internal disulfide bond. The heavy chain typically contains 4 such domains, while the light chain contains 2. The first N-terminal domain of the heavy chain, $V_H$, interacts with the N-terminal domain of the light chain, $V_L$, to produce the binding region of the antibody. Moving towards the C-terminus, the $C_H1$ domain is associated with the constant region of the light chain, $C_L$. The remaining domains on gamma, alpha and delta heavy chains are designated $C_H2$ and $C_H3$, respectively. The mu and epsilon heavy chains contain an additional domain, $C_H4$.

Most heavy chains have a hinge region, consisting of a small number of amino acid residues, between the $C_H1$ and $C_H2$ domains. The hinge is flexible and allows the binding region to move freely relative to the rest of the molecule. At the hinge region are the disulfide bridges which hold the two dimers together, creating the tetramer structural unit.

The hinge region is the point on the molecule most susceptible to the action of protease. Treatment with the protease papain splits the molecule into three fragments, two of which are designated $F_{ab}$ fragments, and the other, the $F_c$ fragment. The $F_{ab}$ fragments each consist of an antigen binding domain and a $C_H1$ domain.

The $F_c$ fragment, which consists of the $C_H2$ and $C_H3$ domains, is the portion of the immunoglobulin molecule that mediates effector functions. Depending upon the heavy chain in the immunoglobulin, a variety of effector functions are possible. These include complement fixation, mediation of antibody dependent cell toxicity, stimulation of B cells, and transport across the placenta. (See, Roitt et al. and Paul, supra). The IgG1 isotype is f particular inter st for us in

the chimeric proteins of the present invention because of its demonstrated effectiveness in complement lysis and antibody-dependent c 11 m diated cytotoxicity (Reichmann et al., Nature, 332:323-327 (1988), which is incorporated herein by reference).

To improve the elimination of the targeted cells, the chimeric protein can be conjugated to a cytotoxic agent to create an immunotoxin. Methods for the production of various immunotoxins is well known in the art. See, generally, "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe, et al., Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982), which is incorporated herein by reference.

The chimeric proteins can also be used to induce anergy in targeted T cells. Recent experiments have shown that, under certain circumstances, anergy or nonresponsiveness can be induced in autoreactive lymphocytes (see, Schwartz, Cell (1989) 1073-1081, which is incorporated herein by reference). In vitro experiments suggest that antigen presentation by MHC Class II molecules in the absence of an unknown co-stimulatory signal induces a state of proliferative non-responsiveness in syngeneic T cells (Quill et al., J. Immunol. (1987) 138:3704-3712, which is incorporated herein by reference).

The immunoglobulin constant region component of the present invention typically comprises the hinge region and the $C_H1$, $C_H2$, and $C_H3$ domains. If mu or epsilon heavy chains are used, a $C_H4$ may be included, as well. As defined here, the constant region component may also comprise a portion of the variable region of the particular immunoglobulin chain, usually less than about 10 amino acids. In certain embodiments, the immunoglobulin component may lack the $C_H1$ domain. An immunoglobulin constant region lacking a $C_H1$ domain is particularly preferred in combination with homodimeric constructs, discussed below. The $C_H1$ domain is believed to be responsible for the phenomenon of "heavy chain toxicity" which is observed when heavy chains are expressed in the absence of their corresponding light chains.

The chimeric molecules of the present invention can exist as either heterodimers or homodimers. Homodimers exist in two classes or f rms, $\alpha/\beta$ homodim rs and $\alpha+\beta$ homodimers. One example of a chimeric molecule of the present invention is illustrated in Figure 2, and comprises constant domains from both the heavy and light chains of the immunoglobulin. Either an MHC II $\alpha$ or $\beta$ chain is linked to each of the immunoglobulin chains. For example, in Figure 2, an MHC II $\alpha$ chain is linked to the heavy chain and the $\beta$ chain is linked to the light chain. The linkage can, of course, be reversed (i.e., $\alpha$ chain to light chain and $\beta$ chain to heavy chain).

Another example of the chimeric molecules of the invention, $\alpha/\beta$ homodimers, comprise a single chain MHC molecule capable of binding the appropriate antigenic peptide and T cell receptor (MHC II $\alpha$ or $\beta$ chain or MHC I heavy chain) linked to an immunoglobulin heavy chain. As illustrated in Figure 3, two $\alpha$ chains are linked to an immunoglobulin heavy chain homodimer. This figure also illustrates a construct lacking $C_H1$ domains, which, as discussed above, are correlated with heavy chain toxicity. The efficacy of single chain MHC components is disclosed and claimed in copending application U.S.S.N. _____ (Attorney Docket No. 14048-16) which is incorporated herein by reference.

Yet anothe example of a chimeric molecule is illustrated in Figure 4. These conjugates are referred to as $\alpha+\beta$ homodimers because each immunoglobulin chain comprises $\alpha$ and $\beta$ chains, or portions thereof, linked together. Typically, the $\alpha$ and $\beta$ chains are attached with a peptide linker as described in Huston et al., Proc. Nat. Acad. Sci. USA 85:5879-5883 (1988) and Chaudary et al., Nature 339:394-397 (1989), both of which are incorporated herein by reference. This technique involves construction of recombinant expression vector encoding the appropriate portions of the $\alpha$ and $\beta$ chains. Preparation and expression of a recombinant DNA constructs is discussed more fully, below. The expression vectors suitable for expressing $\alpha+\beta$ homodimers comprise a sequence encoding the peptide linker placed betw en the two MHC sequences. The

linker preferably exhibits little or no ordered secondary structure and does not substantially interfere with proper folding f the MHC chains. The appropriate linker length is determined by measuring the distance between the C-terminus of one chain and the N-terminus of the other chain. Calculation of the appropriate number of amino acids in the linker is based on a typical peptide unit length of about 0.38 nm.

The chimeric proteins of the present invention may also comprise a protease recognition site between the MHC component and the immunoglobulin component. In some circumstances, the effector and other functions of the immunoglobulin may not be desired. A protease recognition site allows cleavage of the two components and recovery of the purified MHC component. To ensure retention of full MHC function, the sequence of the protease recognition site preferably does not occur in the MHC component. Proteases suitable for the present invention include Factor Xa and collagenase. Treatment of the chimeric protein bound to an affinity chromatography column through the immunoglobulin component can be used to isolate the soluble MHC component in pure form. Suitable columns for this purpose include protein A/G sepharose columns and the like. Alternatively, purified chimeric protein is treated in solution and the MHC component is purified by anti-MHC affinity chromatography.

The MHC and immunoglobulin components of the chimeric proteins may be conjugated by a number of methods. For instance, the linkage may be by way of heterobifunctional cross-linkers, such as SPDP, carbodiimide, glutaraldehyde or the like. Methods for linking protein molecules are well known in the art.

In one aspect, the present invention is directed to recombinant DNA constructs comprising expression cassettes encoding the MHC components and the immunoglobulin components. Generally, the nomenclature used hereafter and the laboratory procedures in recombinant DNA technology described below are those well known and commonly employed in the art. Standard techniques ar used for DNA and RNA isolation, amplification,

and cl ning. Generally enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like are performed according to the manufacturer's specifications. These techniques and various other techniques are generally performed according to Sambrook et al., Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989, which is incorporated herein by reference. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader.

The DNA constructs will typically include an expression control DNA sequence, including naturally-associated or heterologous promoter regions, operably linked to protein coding sequences. The term "operably linked" as used herein refers to linkage of a promoter upstream from one or more DNA sequences such that the promoter mediates transcription of the DNA sequences. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification of the chimeric proteins.

Human MHC and immunoglobulin constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells. For instance, immortalized B cells are suitable for isolating immunoglobulin sequences, e.g., cDNA or genomic DNA. MHC cDNA and genomic clones from a variety of cells have been extensively characterized (see, e.g., Paul, Chapter 17, and Kabat et al., Sequences of Proteins of Immunological Interest, (U.S. Dept. of Health and Human Services, NIH, 1987), which is incorporated herein by reference). Typically, traditional screening of a cDNA library prepared from RNA isolated from appropriate cells is us d. PCR amplification of th desired s quenc s can als be used (See, PCR Protocols, Innis et al., eds. Academic Press,

1990). Suitable source cells for the DNA sequences and host cells for expression and secretion can be obtained fr m a number of sources, such as the American Typ Culture Collection ("Catalogue of Cell Lines and Hybridomas," 6th edition (1988) Rockville, Maryland, U.S.A. and NIGMS Human Genetic Mutant Cell Repository 1990/1991 Catalogue of Cell Lines, 15th edition, NIH Publication No. 91-2011, which are incorporated herein by reference).

The nucleotide sequences used to transfect the host cells can be modified according to standard techniques to yield chimeric molecules with a variety of desired properties. The molecules of the present invention can be readily designed and manufactured utilizing various recombinant DNA techniques well known to those skilled in the art and described in detail, below. For example, the chains can vary from the naturally-occurring sequence at the primary structure level by amino acid, insertions, substitutions, deletions, and the like. These modifications can be used in a number of combinations to produce the final modified protein chain.

The amino acid sequence variants can be prepared with various objectives in mind, including increasing the affinity of the molecule for target T cells, or for facilitating purification and preparation of the chimeric molecule. The modified molecules are also useful for modifying plasma half life, improving therapeutic efficacy, and lessening the severity or occurrence of side effects during therapeutic use. The amino acid sequence variants are usually predetermined variants not found in nature. The variants typically exhibit the same biological activity as naturally occurring MHC molecule. However, the variants and derivatives that are not capable of binding are useful nonetheless (a) as a reagent in diagnostic assays for particular MHC alleles, (b) as agents for purifying anti-MHC antibodies from antisera or hybridoma culture supernatants when insolubilized in accord with known methods, and (c) as immunogens for raising antibodies to MHC alleles s long as at least one MHC epitope remains active.

Polyp ptide fragments comprising nly a portion

(usually at least about 60-80%, typically 90-95%) of the primary structure may be produced. In particular, it is noted that like many genes, the immunoglobulin and MHC genes contain separate functional regions, each having one or more distinct biological activities. Thus, for example, the immunoglobulin component may be modified so as to retain certain functions (e.g., complement fixation activity), while exhibiting lower immunogenicity.

In general, modifications of the genes encoding the chimeric molecule may be readily accomplished by a variety of well-known techniques, such as site-directed mutagenesis (see, Gillman and Smith, Gene 8:81-97 (1979) and Roberts, S. et al., Nature 328:731-734 (1987), both of which are incorporated herein by reference). One of ordinary skill will appreciate that the effect of many mutations is difficult to predict. Thus, most modifications are evaluated by routine screening in a suitable assay for the desired characteristic. For instance, a change in the immunological character of the chimeric molecule can be detected by an appropriate competitive binding assay. The effect of a modification on the ability of the chimeric molecule to bind target T cell receptors can be tested using in vitro cellular assays as described below. Modifications of other properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolysis, or the tendency to aggregate are all assayed according to standard techniques.

Insertional variants of the present invention are those in which one or more amino acid residues are introduced into a predetermined site in the protein and which displace the preexisting residues. For instance, cleavable sequences may be fused to the protein (e.g., sequences form viral proteins) which allow ready affinity chromatographic purification of the fusion protein. Once isolated, the cleavable sequences are removed by treatment with an appropriate protease and the desired GMP-140 molecule is recovered.

Substituti nal variants are those in which at least ne residue has been removed and a different residue ins rt d

in its place. Non-natural amino acid (i.e., amino acids not normally found in native proteins), as well as isosteric analogs (amino acid or otherwise) are also suitable for use in this invention.

Substantial changes in function or immunological identity are made by selecting substituting residues that differ in their effect on the structure of the polypeptide backbone (e.g., as a sheet or helical conformation), the charge or hydrophobicity of the molecule at the target site, or the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in function will be those in which (a) a hydrophilic residue, e.g., serine or threonine, is substituted for (or by) a hydrophobic residue, e.g. leucine, isoleucine, phenylalanine, valine or alanine; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysine, arginine, or histidine, is substituted for (or by) an electronegative residue, e.g., glutamine or aspartine; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine.

Substitutional variants of the subunits also include variants in which functionally homologous (having at least about 70% homology) domains of other proteins are substituted by routine methods for one or more of the MHC domains. Particularly preferred proteins for this purpose are other members of the immunoglobulin superfamily.

Another class of variants are deletional variants. Deletions are characterized by the removal of one or more amino acid residues from the MHC sequence. Typically, the transmembrane and cytoplasmic domains are deleted. Deletions of cysteine or other labile residues also may be desirable, for example in increasing the oxidative stability of the protein. Deletion or substitutions of potential proteolysis sites, e.g., Arg Arg, is accomplished by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

Typically, th transmembrane domain is inactivated by deletion f all the transmembrane domain residues. Inactivation of the membrane binding function is also accomplished by deletion of sufficient residues (not necessarily all the residues) to produce a substantially hydrophilic hydropathy profile at this site or by substituting with heterologous residues which accomplish the same result.

Glycosylation variants are included within the scope of this invention. They include variants completely lacking in glycosylation (unglycosylated) and variants having at least one less glycosylated site than the native form (deglycosylated) as well as variants in which the glycosylation has been changed. Included are deglycosylated and unglycosylated amino acid sequence variants, deglycosylated and unglycosylated subunits having the native, unmodified amino acid sequence. For example, substitutional or deletional mutagenesis is employed to eliminate the N- or O-linked glycosylation sites of the protein, e.g., the asparagine residue is deleted or substituted for by another basic residue such as lysine or histidine. Alternatively, flanking residues making up the glycosylation site are substituted or deleted, even though the asparagine residues remain unchanged, in order to prevent glycosylation by eliminating the glycosylation recognition site. Additionally, unglycosylated subunits which have the amino acid sequence of the native subunits are produced in recombinant prokaryotic cell culture because prokaryotes are incapable of introducing glycosylation into polypeptides.

Glycosylation variants are conveniently produced by selecting appropriate host cells or by in vitro methods. Yeast, for example, introduce glycosylation which varies significantly from that of mammalian systems. Similarly, mammalian cells from a different species (e.g., hamster, murine, insect, porcine, bovine or ovine) or tissue than the GMP-140 source are routinely screened for the ability to introduce variant glycosylation as characterized for example by elevated levels of mannose or variant ratios of mannose, fucose, sialic acid, and other sugars typically found in

mammalian glycoproteins. In vitro processing of the subunit typically is accomplish d by enzymatic hydrolysis, e.g., neuraminidase digestion.

As stated previously, the nucleotide sequences encoding the MHC and immunoglobulin components will be expressed in hosts after the sequences have been operably linked to an expression control sequence (i.e., positioned to ensure the translation of the structural gene) along with other sequences (e.g., enhancers, polyadenylation sites, etc.) necessary for efficient transcription and translation of the desired sequences. This collection of sequences is referred to here as an "expression cassette" which is typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors comprising the expression cassette will contain selection markers, e.g., tetracycline or neomycin, to permit detection of those cells transformed with the desired sequences (see, e.g., U.S. Patent 4,704,362, which is incorporated herein by reference).

In general, prokaryotes are used for cloning the chimeric protein nucleotide sequences. E. coli is particularly useful for cloning the nucleotide sequences of the present invention. Other microbial hosts suitable for use include bacilli, such as Bacillus subtilus, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast may also be used for expression. Saccharomyces is a preferred host, with suitable vect rs having expressi n contr l sequences, an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase 2, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

When constructing vectors for use in yeast, the plasmid YRp7 can be used (Stinchcomb, et al., Nature, 282: 39 (1979), which is incorporated by reference). This plasmid contains the trp1 gene which is a selectable marker for a mutant strain which is unable to grow on media lacking tryptophan. The presence of the trp1 gene allows transformed mutant cells to grow on selective media and to be identified.

Mammalian tissue cell culture will typically be used to produce the polypeptides of the present invention (see, Winnacker, "From Genes to Clones," VCH Publishers, N.Y., N.Y. (1987), which is incorporated herein by reference). Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting intact immunoglobulins or MHC molecules have been developed in the art, and include the CHO cell lines, various COS cell lines, HeLa cells, myeloma cell lines such as SP2/0 or lymphoma lines such as BW5147, etc. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen, C. et al., Immunol. Rev. 89:49-68 (1986) and Kriegler, Gene Transfer and Expression, (Stockton Press 1990), which are incorporated herein by reference), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, Adenovirus, Bovine Papilloma Virus, hCMV early enhancer/promoter (Boshart et al., Cell 41:521-530 (1985)), SRα

(Takebe et al., Mol. and Cell Biol., 8:486-472 (1988)) and the like.

Eukaryotic transcription can be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting sequences of between 10 to 300bp that increase transcription by a promoter. Enhancers can effectively increase transcription when either 5' or 3' to the transcription unit. They are also effective if located within an intron or within the coding sequence itself. Typically, viral enhancers are used, including SV40 enhancers, cytomegalovirus enhancers, polyoma enhancers, and adenovirus enhancers. Enhancer sequences from mammalian systems such as the mouse immunoglobulin heavy chain enhancer are also commonly used.

Mammalian expression vector systems will also typically include a selectable marker gene. Examples of suitable markers include, the hypoxanthine-guanine phosphoribosyl transferase gen (HGPT), the thymidine kinase gene (TK), or various prokaryotic genes conferring drug resistance. Amplifiable marker genes, such as dihydrofolate reductase gene (DHFR), may also be used (see, generally, Kriegler, supra). Examples of prokaryotic drug resistance genes useful as markers include genes conferring resistance to neomycin, G418, and hygromycin.

As discussed above, the chimeric proteins may also comprise protease recognition sites between the MHC and immunoglobulin components. Thus, nucleotides encoding the appropriate protease recognition site will be included. For Factor Xa, the recognition site is Ile(Glu/Asp)GlyArg. For collagenase, the recognition site is ProLeuGlyPro(D-Arg).

The vectors containing the nucleotide segments of interest (e.g., an expression cassette comprising the MHC chain and immunoglobulin encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment,

cationic liposomes, or electroporati n may be used for other cellular hosts. Other methods used to transform mammalian cells include the use of Polybrene, protoplast fusi n, microprojectiles and microinjection. See, generally, Sambrook et al., supra.

Once expressed, the whole chimeric proteins, or individual chains of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity and fraction column chromatography, gel electrophoresis and the like, (See, generally, Scopes, R., Protein Purification, Springer-Verlag, N.Y. (1982), which is incorporated herein by reference.) Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically or in developing and performing assay procedures, immunofluorescent stainings, and the like. (See, generally, Immunological Methods, Vols. I and II, Eds. Lefkovits and Pernis, Academic Press, New York, N.Y. (1979 and 1981), which are incorporated herein by reference.)

Therapeutic uses of the chimeric molecules of the present invention require identification of the MHC haplotypes and antigens useful in treating a particular disease. The present invention is particularly suitable for treatment of autoimmune disease. Based on knowledge of the pathogenesis of human autoimmune disease and the results of studies in relevant animal models, one skilled in the art can readily identify and isolate the MHC haplotype and autoantigen associated with a variety of autoimmune diseases.

A number of specific autoimmune diseases have been correlated with specific MHC types. Methods for identifying which alleles, and subsequently which MHC encoded polypeptides, are associated with an autoimmune disease are well known in the art. For example, the association between an MHC antigen and an autoimmune disease can be determined based upon genetic studies. The methods for carrying out these studies were known to th se skilled in th art, and information on all known HLA disease associati ns in humans is maintain d in the HLA and

Disease Registry in Copenhagen. Methods for identifying appr priate MHC haplotypes are fully discussed in the following copending, and commonly assigned, U.S. patent applications: 07/576,084, filed August 30, 1990 and 07/367,751, filed June 21, 1989, which are incorporated herein by reference.

Once the allele which confers susceptibility to the specific autoimmune disease is identified, the MHC antigen encoded by the allele is also identifiable. As an example, over 90% of rheumatoid arthritis patients have a haplotype of DR1, DR4 (Dw4) or DR4 (Dw14). Hence, the chimerics of the present invention used for treatment or diagnosis of an individual with rheumatoid arthritis would include a MHC II component encoded by the DR, DQ or DP gene of the DR4 haplotype which contains the Dw4 or Dw14 DR allelic determinant.

Contemporary knowledge of the autoantigens associated with particular autoimmune diseases is extensive. Identified autoantigens include acetylcholine receptor in myasthenia gravis, myelin basic protein in multiple sclerosis, mitochondrial dihydrolipoamide acyltransferase in primary biliary cirrhosis, type II collagen in rheumatoid arthritis, thyroglobulin in autoimmune thyroiditis, S antigen in autoimmune uveitis, and desmoplakin I in paraneoplastic pemphigus. In those autoimmune diseases that have been extensively studied, small autoantigenic peptide fragments (epitopes) of the macromolecular autoantigen have been shown to be recognized by a defined subset of helper T cells (Livingstone et al., Ann. Rev. Immunol. 5:477-501 (1987), which is incorporated herein by reference).

Once a macromolecular autoantigen has been identified as the target of an autoimmune response, several published techniques may be used to identify and characterize the epitope recognized by the target T cell. The assay methods typically use antigenic fragments generated by enzymatic digestion of the whole autoantigen or by cloning and expression of fragments of the gene encoding the autoantigen. When the amino acid sequence f th autoantigenic peptide fragm nt is known, sets of verlapping peptides ar then synthesized. These assays

identify epitopic sequences by the ability of the fragments or synthetic peptides to stimulate disease associated T cell clones or hybridomas in syngeneic antigen-presenting systems (see, e.g., Watts et al., Ann. Rev. Immunol. 5: 461-475 (1987); Lamb et al. EMBO J. 6:1245-1249 (1987); Berkower et al., J. Immunol. 136:2498-2502 (1986); and Townsend et al., Cell 44:959-968 (1986); all of which are incorporated herein by reference).

Finally, it is frequently desirable to charge the chimeric molecule with the appropriate autoantigenic peptide, identified by the methods described above. This is typically done by incubating the purified chimeric with excess peptide at a low or high pH so as to open the antigen binding pocket (see, Harding et al., Proc. Nat. Acad. Sci. USA 88:2740-2744 (1991), which is incorporated herein by reference). The peptide is thus noncovalently linked with the antigen binding pocket of the MHC component. The antigenic peptide can also be covalently bound using standard procedures such as photo-affinity labelling, (see e.g., Hall et al., Biochemistry 24:5702-5711 (1985), which is incorporated herein by reference). This method has previously been shown to be effective in covalently binding antigen in peptides to antigen binding pockets. See, e.g., Leuscher et al., J. Biol. Chem., 265:11177-11184 (1990) and Wraith et al., Cell, 59:247-255 (1989), which are incorporated herein by reference.

The chimeric molecules of the invention can be assayed using an in vitro system or using an in vivo model. In the in vitro system, the molecule is incubated with peripheral blood T cells from subjects immunized with, or showing immunity to, the protein or antigen responsible for the condition associated with the peptide of the complex. The successful molecules will eliminate (or induce anergy in) syngeneic T cells as measured in the assays described above.

In the in vivo system, T cells that proliferate in response to the isolated epitope or to the full length antigen in the presence of antigen presenting cells are cloned. The clones are injected into histocompatible animals which have not

be n immunized in order to induce the autoimmune disease. Symptoms r lated to the relevant complex should ameliorate or eliminate th symptoms of the disease.

Chimeric proteins of the present invention can find a wide variety of in vitro and in vivo utilities. By way of example, they can be used to prepare purified MHC compositions. For instance, a protein A/G sepharose column can be used to bind the immunoglobulin component of a chimeric protein of the present invention. Treatment of the bound chimeric protein with the appropriate protease will release the soluble MHC component in a pure form. Alternatively, the chimeric protein can be protease treated in solution and the MHC component can be isolated using the general methods described above for purifying chimeric proteins. For instance, an anti-MHC antibody column can be used to obtain a pure MHC component preparation.

The chimeric proteins can also be used in a variety of in vivo applications, such as treating or monitoring autoimmune diseases. In vitro uses include, diagnostic applications, T cell typing, isolating or labeling specific cells, and the like. For any of these purposes, the chimeric proteins may be labeled or unlabeled.

Unlabeled chimeric proteins can be used in combination with labeled antibodies that are reactive with the immunoglobulin portion of the molecule. Antibodies specific for human immunoglobulin constant regions are well known in the art. See, generally, Harlow and Lane, Antibodies: A Laboratory Manual (1988). Labeled protein A or protein G may also be used for this purpose. These proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species. See, generally Kronval, et al., J. Immunol., 111:1401-1406 (1973), and Akerstrom, et al., J. Immunol., 135:2589-2542 (1985), all of which are incorporated herein by reference.

Alternatively, the chimeric protein can b directly labeled. A wide variety of labels may be employed, such as

radionuclides, fluorescers, enzyms, enzyme substrates, enzym cofactors, enzyme inhibitors, chemiluminescent compounds, bioluminescent compounds, etc. Those of ordinary skill in the art will know of other suitable labels for binding to the chimeric protein, or will be able to ascertain such using routine experimentation. The binding of these labels can be done using standard techniques common to those of ordinary skill in the art.

In using the chimeric proteins of the invention for the in vivo detection of cells associated with a particular disease or condition, the detectably labeled chimeric protein is given in a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled protein is administered in sufficient quantity to enable detection of cells having the receptor for which the MHC component is specific. The concentration of detectably labeled protein which is administered should be sufficient such that the binding to those cells having the receptor is detectable compared to the background signal. Further, it is desirable that the detectably labeled protein be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio. As a rule, the dosage of detectably labeled protein for in vivo diagnosis will vary depending on such factors as age, sex and extent of disease of the individual.

For in vivo diagnostic imaging, radioisotopes are typically used. The type of detection instrument available is a major factor in selecting the radioisotope used. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for in vivo diagnosis is that the half-life of the radioisotope be long enough so that it is still detectable at the time of maximum uptake by the target, but short enough so that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for in vivo imaging will lack a particle emission, but produce a large number of photons in the 140-250

keV range, which may be readily detected by conv ntional gamma cameras.

For in vivo diagnosis, radioisotopes may be bound to the protein either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to proteins are the bi-functional chelating agents such as diethylenetriaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules.

The proteins of the invention can also be labeled with a paramagnetic isotope for purposes of in vivo diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI.

The proteins of the present invention can be used to monitor the course of amelioration of an immune response mediated disorder (such as autoimmunity) in an individual. Thus, by measuring the increase or decrease in the number of targeted T cells, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the immune response mediated disorder is effective.

For therapeutic uses, a variety of pharmaceutical compositions comprising the claimed proteins can be prepared. Pharmaceutical compositions comprising the proteins are useful for, e.g., parenteral administration, i.e., subcutaneously, intramuscularly or intravenously. In addition, a number of new drug delivery approaches are being developed. The pharmaceutical compositions of the present invention are suitable for administration using these new methods, as well. See, Langer, Science 249:1527-1533 (1990), which is incorporated herein by reference.

The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acc ptabl carrier, preferably an aque us carrier. A variety of aqueous carriers can be used,

e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These c mpositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of the chimeric protein in these formulations can vary widely, i.e., from less than about 1 μg/ml, usually at least about 0.1 mg/ml to as much as 10 - 100 mg/ml by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made up to contain 1 ml sterile buffered water, and 0.1 mg of chimeric protein. A typical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 10 mg of chimeric protein. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 17th Ed., Mack Publishing Company, Easton, Pennsylvania (1985), which is incorporated herein by reference.

The chimeric proteins of this invention can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins and commonly used lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of activity loss and that use levels may have to be adjusted to compensate.

The c mpositions containing the present chimeric proteins or a c cktail thereof can be administered f r the

prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already affected by the particular disease, in an amount sufficient to cure or at least partially arrest the disease process and its complications. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend upon the severity of the disease and the general state of the patient's own immune system, but generally range from about 0.01 to about 1000 mg of chimeric protein per dose, with dosages of from about 10 to about 100 mg per patient being more commonly used.

In prophylactic applications, compositions containing the chimeric proteins or a cocktail thereof are administered to a patient not already in a disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts again depend upon the patient's state of health and general level of immunity, but generally range from 0.01 to 1000 mg per dose, especially about 10 to about 100 mg per patient.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the chimeric proteins of this invention sufficient to effectively treat the patient.

Kits can also be supplied for therapeutic or diagnostic uses. Thus, the subject composition of the present invention may be provided, usually in a lyophilized form in a container. The proteins, which may be conjugated to a label or toxin, or unconjugated, are included in the kits with buffers, such as Tris, phosphate, carbonate, etc., stabilizers, biocides, inert proteins, e.g., serum albumin, or the like, and a set of instructions for use. Generally, these materials will be present in less than about 5% wt. based on the amount of active chimeric protein and usually present in total amount of at least about 0.001% wt. based on the protein

concentrati n. Frequently, it will be desirable t include an inert extender r excipient t dilute the active ingredients, where the excipient may be present in from about 1 to 99% wt. of the total composition. Where a second antibody capable of binding to the chimeric protein is employed in an assay, this will usually be present in a separate vial. The second antibody is typically conjugated to a label and formulated in an analogous manner with the antibody formulations described above.

The following example is offered by way of illustration, not by way of limitation.

## EXAMPLE I

This example shows the construction of an expression vector capable of directing expression of a chimeric protein in an appropriate mammalian cell.

Nucleotide sequences from the Class II MHC gene, HLA DR4 (DW4), are obtained using standard procedures from GMD6821a cells, which can be obtained from NIGMS Human Genetic Mutant Cell Repository, supra.

A unique restriction site Xba1 followed by a Kozak consensus ribosomal binding site (Kozak et al., Nuc. Acids. Res., 12:857-872 (1984), which is incorporated herein by reference) is added to the 5' end of an isolated Class II MHC gene immediately upstream of the translational start codon, ATG, of the leader peptide.

Based upon the hydrophobicity profile of the $\alpha$ and $\beta$ chains (Kyte and Doolittle, J. Mol. Biol., 157:105-132 (1982), incorporated herein by reference), nucleotides encoding the appropriate protease recognition site flanked by nucleotides encoding ($Gly_4$) are inserted immediately upstream of the hydrophobic transmembrane domain. For Factor Xa, the amino acid sequence is Ile(Glu/Asp)GlyArg, for collagenase, ProLeuGlyPro(D-Arg).

For the use of genomic versions of the IgG1 constant domains, the splice d nor sequence C/AAGGTA/GAGT and the XbaI restricti n site are placed immediately downstream of the

[($Gly_4$)protease recognition($Gly_4$)] site. The splice donor is plac d in such a manner that the translational reading frame of the MHC II/IgG hybrid mRNA is not altered.

For the use of cDNA versions of the IgG1 constant domains, the splice donor will be replaced with an XbaI or another restriction site that has the minimum effect on the amino acid sequence of the chimeric protein.

The final genes are excised with XbaI (or a combination of XbaI and a second restriction enzyme) and inserted into expression vectors. The expression vectors are depicted schematically in Figure 5. An expression vector will comprise sequences encoding either the $\alpha$ or the $\beta$ MHC chain and the immunoglobulin constant region gene. Figure 5a shows a vector comprising immunoglobulin cDNA and Figure 5b shows the construct with genomic immunoglobulin DNA. The important components of the vectors include the following. An ampicillin resistance gene and bacterial origin of replication derived from the pUC series of plasmids is included to allow cloning in a bacterial host. To allow selection of transformed eukaryotic cells, a drug resistance gene (mycophenolic $acid^R$) is included. Transcription of this gene is driven by an SV40 early/late promoter followed by a SV40 early/late poly-adenylation signal. A strong enhancer/promoter (hCMV early enhancer/promoter) drives expression of the inserted chimeric protein gene.

For expression vectors comprising genomic IgG1 nucleotide sequences, a splice acceptor sequence, T/CT/CT/CT/CT/CT/CT/CT/CT/CT/CT/CNC/TAGG/A, is used, followed by the sequence encoding either the rearranged genomic heavy or light chain IgG1 constant region (Hieter et al., Cell, 22:197-207 (1980); Ellison et al., Nuc. Acids Res., 10:4071-4079 (1982); and Takahashi et al., Cell, 29:671-679 (1982), all of which are incorporated herein by reference). The splice acceptor will be placed in such a manner that the translational reading frame of the hybrid mRNA will be unaffected.

For expression vectors comprising IgG1 cDNA, the mature cDNA sequences f the heavy or light chain IgG c nstant regions are linked directly to the appropriate MHC sequence.

Following the constant region coding sequences will be either the endogenous polyadenylation signal (genomic) or a heterologous signal (cDNA).

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A composition comprising a chimeric molecule which selectively binds a T cell receptor, the chimeric molecule comprising an MHC component linked to an immunoglobulin constant region component, wherein the MHC component comprises an antigen binding pocket bound to an autoantigenic peptide.

2. The composition of claim 1 wherein the MHC component consists essentially of an extracellular region of an MHC molecule.

3. The composition of claim 1 wherein the MHC component is Class II.

4. The composition of claim 1 wherein the MHC component is human.

5. The composition of claim 1 further comprising a protease recognition site between the MHC component and the immunoglobulin constant region component.

6. The composition of claim 5 wherein the protease is Factor Xa or collagenase.

7. The composition of claim 1 wherein the immunoglobulin constant region component is a human IgG1 isotype.

8. The composition of claim 1 wherein the immunoglobulin constant region component consists essentially of a hinge region, a $C_H2$ domain, and a $C_H3$ domain.

9. The composition of claim 1 wherein the immunoglobulin constant region component fixes compelment.

10. The composition of claim 1 wherein the immunoglobulin constant region component mediates antibody dependent cell cytotoxicity.

11. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a chimeric molecule which selectively binds a T cell receptor, the chimeric molecule comprising an MHC component linked to an immunoglobulin constant region component, wherein the MHC component comprises an antigen binding pocket bound to an autoantigenic peptide.

12. A method of treating an autoimmune disease in a patient comprising administering to the patient a therapeutically effective dose of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a chimeric molecule which selectively binds a T cell receptor, the chimeric molecule comprising an MHC component linked to an immunoglobulin constant region component, wherein the MHC component comprises an antigen binding pocket bound to an autoantigenic peptide.

13. A method of claim 12, wherein the pharmaceutical composition is administered intravenously.

14. A method of claim 12 wherein the autoimmune disease is rheumatoid arthritis or multiple sclerosis.

15. A recombinant expression cassette comprising a promoter sequence operably linked to a first nucleotide sequence encoding an MHC protein chain and a second nucleotide sequence encoding an immunoglobulin constant region protein chain.

16. A recombinant expression cassette of claim 15 wherein the first nucleotid sequence encodes an extracellular region of a Class II MHC $\alpha$ chain or a Class II MHC $\beta$ chain.

17. A recombinant expression cassette of claim 15 wherein the second nucleotide sequence encodes an immunoglobulin constant region protein chain comprising $C_\gamma 1$ or $C_K$.

18. A recombinant expression cassette of claim 15 wherein the second nucleotide sequence is cDNA.

19. A recombinant expression cassette of claim 15 further comprising a third nucleotide sequence positioned between the first and second nucleotide sequences, the third nucleotide sequence encoding a protease recognition site.

20. A method of preparing a chimeric protein, the method comprising transforming a host cell with a recombinant construct having the expression cassette of claim 15 and recovering the chimeric protein from a culture of the host cell.

21. The method of claim 20 wherein the host cell is a mammalian cell.

22. The method of claim 21 wherein the host cell is a CHO, BW5147, or SP2/0 cell.

23. A method of purifying a soluble MHC molecule, the method comprising the steps of

contacting an affinity column with a composition comprising a chimeric molecule having an an MHC component linked to an immunoglobulin constant region component via an amino acid sequence having a protease recognition site, wherein the affinity column specifically binds the immunoglobulin constant region;

treating the column with a protease capable of selectively cleaving the chimeric protein at the protease rec gnition site, thereby releasing the MHC component from the c lumn.

24. A method of claim 23 wherein the affinity column is a Protein A/G-Sepharose column.

25. A method of claim 23 wherein the MHC component comprises an extracellular portion of an $\alpha$ and a $\beta$ protein chain from a Class II MHC molecule.

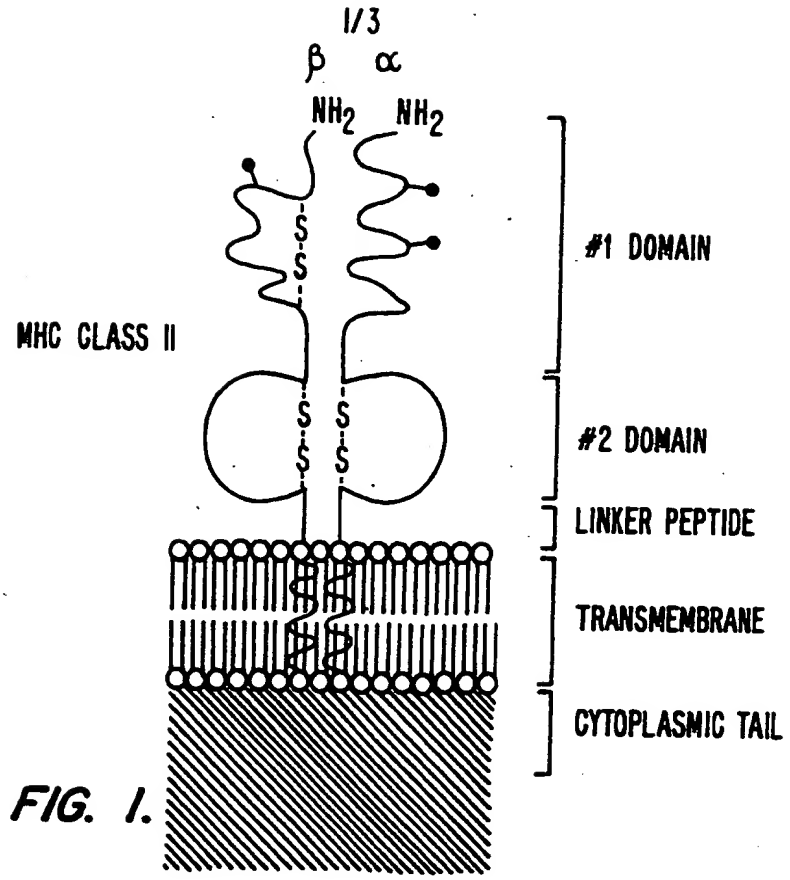
β α
NH2 NH2
MHC CLASS II
#1 DOMAIN
#2 DOMAIN
LINKER PEPTIDE
TRANSMEMBRANE
CYTOPLASMIC TAIL

FIG. 1.

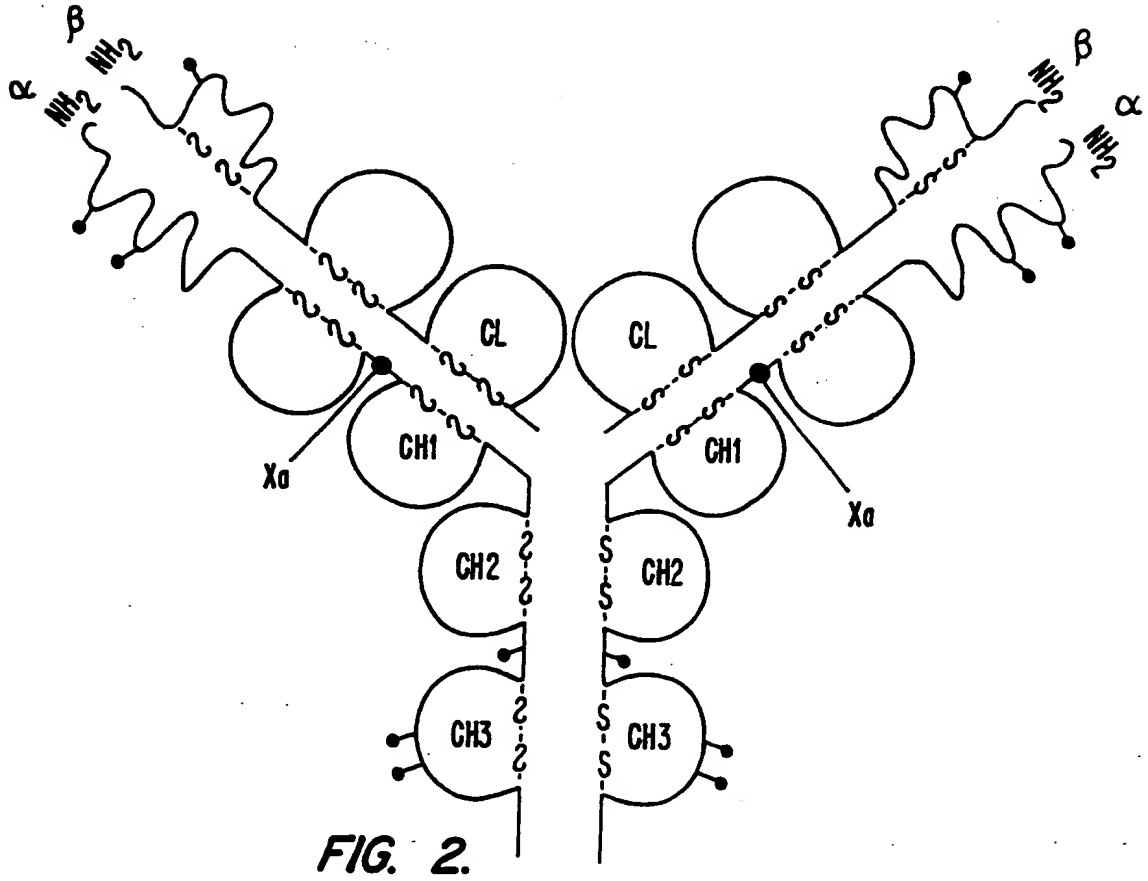
β
NH2
α
NH2
CL
CH1
Xa
CH2
CH3
S-S

FIG. 2.

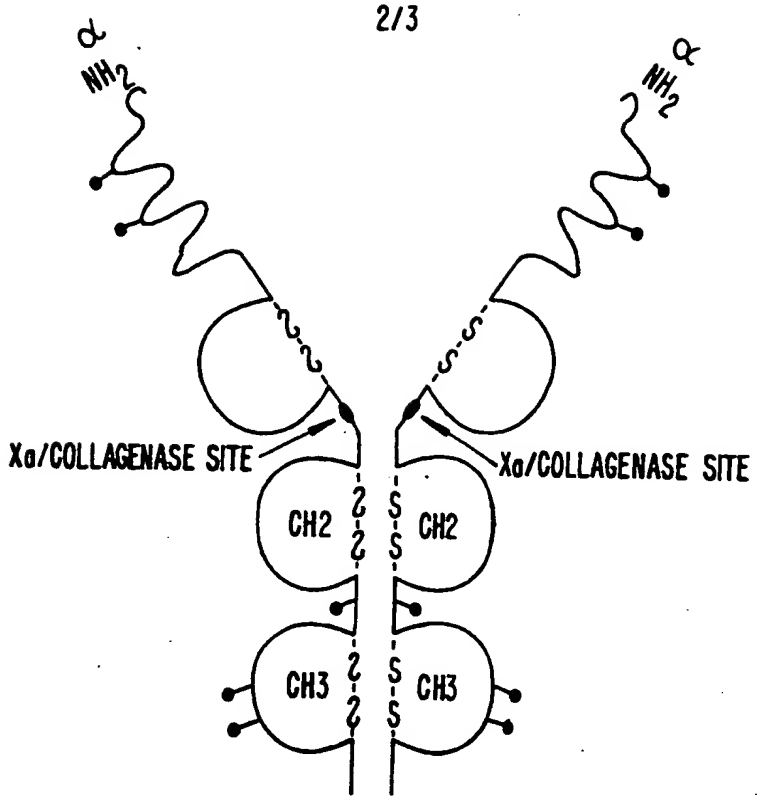
α
NH2
α
NH2
Xa/COLLAGENASE SITE
Xa/COLLAGENASE SITE
CH2
CH2
CH3
CH3

FIG. 3.

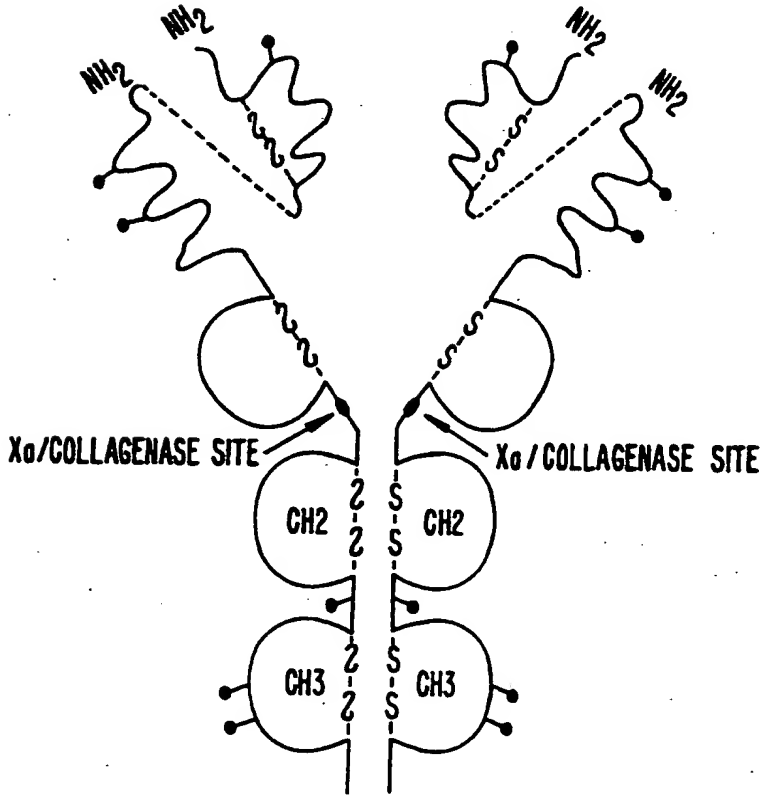
NH2
NH2
NH2
NH2
Xa/COLLAGENASE SITE
Xa / COLLAGENASE SITE
CH2
CH2
CH3
CH3

FIG. 4.

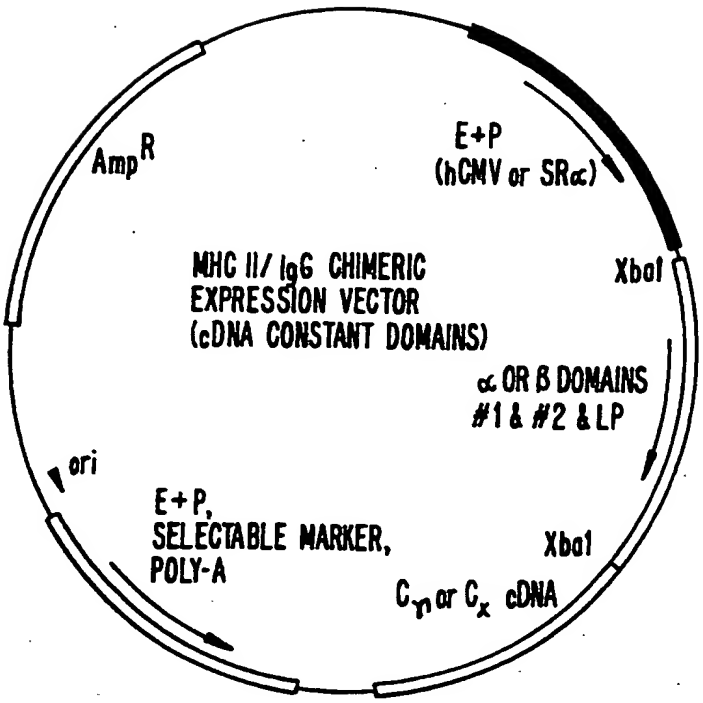
Amp^R
E+P
(hCMV or SRα)
MHC II/ IgG CHIMERIC
EXPRESSION VECTOR
(cDNA CONSTANT DOMAINS)
Xba1
α OR β DOMAINS
#1 & #2 & LP
ori
E+P,
SELECTABLE MARKER,
POLY-A
Xba1
C_γ or C_χ cDNA

FIG. 5a.

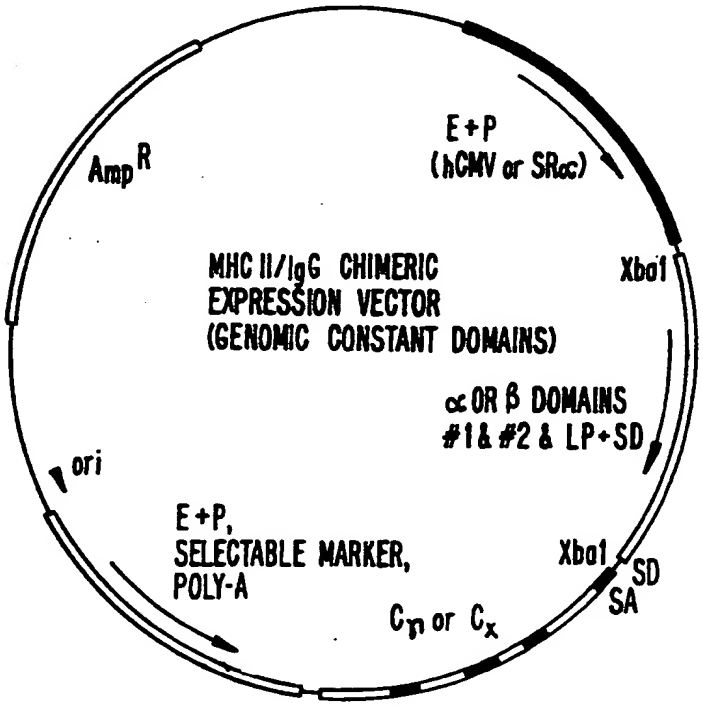
Amp^R
E+P
(hCMV or SRα)
MHC II/IgG CHIMERIC
EXPRESSION VECTOR
(GENOMIC CONSTANT DOMAINS)
Xba1
α OR β DOMAINS
#1& #2 & LP+SD
ori
E+P,
SELECTABLE MARKER,
POLY-A
Xba1
SD
SA
C_γ or C_χ

FIG. 5b.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10030

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 5/10; C12P 21/00; A61K 37/00, 39/00
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/85.91; 435/69.1, 69.7, 91; 530/387.3, 388.22, 388.73, 388.75; 536/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG: MEDLINE, BIOSIS, WPI, CA
search terms: immunoglobulin, major histocompatibility complex, mhc, chimer?, hybrid

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,Y | US,A, 5,130,297 (Sharma et al.) 14 July 1992, see entire document. | 1-25 |
| Y | Nature, Volume 337, issued 09 February 1989, D.J. Capon et al., "Designing CD4 Immunoadhesins for AIDS Therapy", pages 525-530, see entire document. | 1-25 |
| Y | ICSU Short Reports, issued February 1988, Nilsson et al., "Using Genetics to Facilitate Downstream Processing", pages 122-123, see entire document. | 5,6,19,23-25 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be part of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>09 FEBRUARY 1993 | Date of mailing of the international search report<br>19 FEB 1993 |
| Name and mailing address of the ISA/<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. NOT APPLICABLE | Authorized officer<br>PHILLIP GAMBEL<br>Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10030

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/85.91; 435/69.1, 69.7, 91; 530/387.3, 388.22, 388.73, 388.75; 536/27